BIOLOGY OF REPRODUCTION **60,** 115–118 (1999)

Testicular Morphology and Function in Boars Differing in Concentrations of Plasma Follicle-Stimulating Hormone[1]

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ABSTRACT

The objective of this study was to evaluate morphological characteristics and testicular function of boars with different endogenous concentrations of FSH. Boars were selected at 6 mo of age on the basis of mean FSH concentrations in plasma collected at 4, 5, and 6 mo of age. Boars were classified within half-sibling families based on whether they had high concentrations of FSH (HiFSH, $>$ 500 ng/ml, n = 9) or low concentrations (LoFSH, $<$ 500 ng/ml, n = 7). At 14.5 mo, testes were collected, fixed, sectioned at 1 μm, and evaluated for morphological characteristics. Boars with LoFSH had larger ($p < 0.01$) testicular and epididymal weights than boars with HiFSH, greater ($p < 0.01$) daily sperm production per gram of testis, and greater total daily sperm production per boar. Testes of boars with LoFSH had a greater ($p < 0.03$) volume percentage of seminiferous tubules, a lesser percentage ($p < 0.03$) of Leydig cells, and a somewhat lesser ($p = 0.06$) percentage of vascular structures than testes of boars with HiFSH. Testes of boars with LoFSH had greater ($p < 0.01$) total tubule volume and tubule length than testes of boars with HiFSH. There were no differences ($p > 0.70$) in volume, diameter, or total number of Leydig cells or in total interstitial volume in testes ($p > 0.41$) of these two groups. Production of testosterone in vitro per paired testis and per million Leydig cells was not different ($p > 0.65$) between boars with HiFSH or LoFSH. Greater concentrations of FSH in blood plasma were negatively associated with development of seminiferous tubules and spermatogenic efficiency, whereas Leydig cell development was not different in boars of these two groups.

INTRODUCTION

Boars of the Meishan breed reach puberty at an earlier age (56–84 days) than boars of European breeds (120–180 days) [1, 2]. Compared to White Composite and Duroc boars, Meishan boars have greater concentrations of FSH, LH, and androgens in blood plasma during early postnatal, pubertal, and postpubertal development [2–4]. Furthermore, mature Meishan boars have lower paired testicular weights, a greater percentage of interstitial structures, larger Leydig cells, a lesser percentage of seminiferous tubules, less Sertoli cells per gram of testicular tissue, and lower total daily sperm production (TDSP) compared to Duroc [3] and White Composite boars [2, 5, 6].

In many species, elevated plasma FSH concentrations during prepubertal development are associated with increased growth of seminiferous tubules [7–9]. In contrast to expectations, concentrations of FSH in blood plasma within lines of postpubertal boars were negatively correlated with testicular size [10]. Thus, boars with greater concentrations of FSH had greatly reduced TDSP on the basis of less testicular mass than boars with lower concentrations of plasma FSH. The objective of the present study was to evaluate testicular morphology in boars (Meishan × White Composite) that had different endogenous concentrations of FSH in blood plasma. Additionally, basal testosterone production was assessed in vitro to determine whether the endogenous concentrations of gonadotropins that differed in these two groups of boars altered in vitro Leydig cell function.

MATERIALS AND METHODS

Sixteen boars of Meishan × White Composite breeding from three sires were selected at 6 mo of age on the basis of mean FSH concentrations from plasma collected at 4, 5, and 6 mo of age. These 1/2 MS × 1/2 WC boars, which originated by reciprocal matings (3/4 MS × 1/4 WC to 1/4 MS × 3/4 WC), were classified within half-sibling families based on whether they had greater (HiFSH, $>$ 500 ng/ml, n = 9) or lesser (LoFSH, $<$ 500 ng/ml, n = 7) concentrations of FSH and were evaluated after they reached 1 yr of age. Within each sire family there was at least one boar in the HiFSH and one in the LoFSH group. Boars were housed individually, and anesthesia was induced with halothane to facilitate insertion of indwelling jugular catheters a minimum of 4 days before sequential blood samples were collected [11]. To characterize concentrations of hormones in blood plasma, samples were collected every 20 min for 2 h.

Boars were subsequently stunned electrically and exsanguinated; testes were then collected. At slaughter, boars in the two groups were of similar age (438 ± 2.7 vs. 435 ± 2.2 days) and body weight (148.5 ± 5.8 vs. 147.2 ± 4.6 kg). The weights of each testis and epididymis were recorded, and parenchymal tissue from the right testis was incubated according to previously described procedures [12]. Briefly, random samples of parenchymal tissue were dissected free and minced, and approximately 400-mg aliquots were incubated in 25-ml Erlenmeyer flasks with 5 ml of tissue culture medium 199 (TC199) buffered with Hepes at a final concentration of 25 mM. After a 1-h preincubation period in a shaking water bath (35°C) and in an atmosphere of 95% O_2:5% CO_2, medium was carefully decanted and replaced with 5 ml of TC199 (25 mM Hepes). Six flasks of tissue were incubated for each boar. Aliquots (250 μl) were collected immediately after the preincubation period

Accepted September 1, 1998.
Received July 7, 1998.
[1]Research supported by the Nebraska Agricultural Division and UNL Center for Biotechnology. Published as Paper No. 12266 Journal Ser. Nebraska Agr. Res. Div. Mention of names is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the same by USDA implies no approval of the product to the exclusion of others that may also be suitable.
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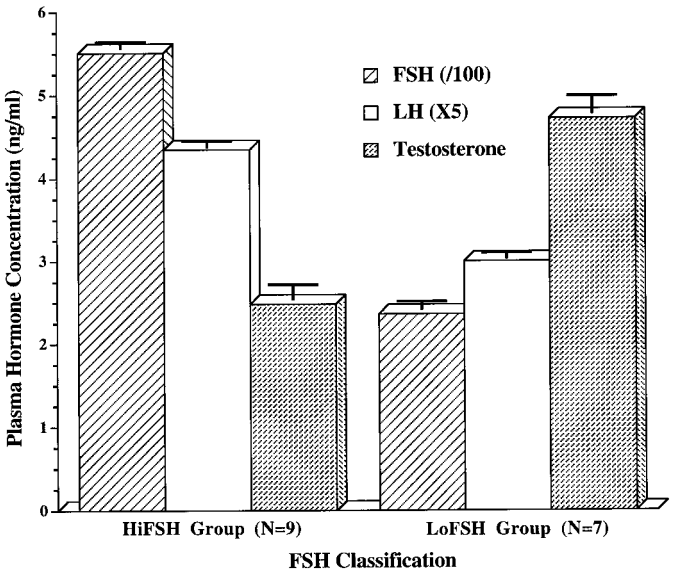

FIG. 1. Mean concentrations at 14.5 mo of age of FSH, LH, and testosterone in blood plasma of Meishan × White Composite boars that were selected for differences in FSH concentrations at 6 mo of age. Concentrations of all three hormones differed ($p < 0.01$) between groups throughout the 2-h sampling period. Reference preparations were USDA pFSH-B1 and pLH-B1.

(Time 0) and at 30, 60, and 120 min; they were immediately frozen on dry ice and kept at −20°C until assayed directly for testosterone by RIA without extraction [12].

Concentrations of testosterone in blood plasma were determined by RIA [12, 13]. Concentrations of LH were determined [14] using anti-porcine LH (AFP 151031194) and porcine LH (AFP 10714B) for iodination, which were provided by Dr. A.F. Parlow. The reference preparation was USDA porcine (p)LH-B1. All plasma samples that were evaluated for LH and testosterone were included within a single assay for each respective hormone; the minimum sensitivities were 0.3 and 0.2 ng/ml, and the intraassay coefficients of variation were 8.1% and 10.1%, respectively. Concentrations of FSH in plasma were determined by RIA using anti-ovine FSH (AFP C5288113) following a protocol previously described [15, 16]. Minimum sensitivity was 20 ng/ml, and the interassay coefficients of variation were 16% and 13% for pools of sera that assayed, respectively, 272 and 1370 ng FSH/ml.

The left testis was perfused with 3% glutaraldehyde and 1% formaldehyde in 0.1 M cacodylate buffer. Tissue was then cut into small pieces (5 mm × 5 mm), rinsed in 0.1 M cacodylate, postfixed in 1% osmium tetroxide in 0.1 M cacodylate, dehydrated in a graded series of ethanol, and embedded in Araldite 502 (Ladd Research, Burlington, VT). Sections (1 μm) were cut and stained with 1% toluidine blue in 1% sodium tetraborate and 0.5% Fuchsin solutions [12]. The morphology of three sections per boar was evaluated via brightfield microscopy using computerized morphometric planimetry [17] (Bioquant VI system; R & M Biometrics Corp., Nashville, TN). Rate of sperm production was evaluated as number of homogenization-resistant spermatids [18, 19].

Statistical analyses were performed using a one-way ANOVA utilizing General Linear Models procedures of the Statistical Analysis System [20]. The model for repeated measurements of hormone concentrations included group, group × time, and sire. Morphological data were also evaluated for sire effects. Data are presented as least-squares means ± SE.

TABLE 1. Body weight, testicular weight, daily sperm production, and TDSP in boars of the HiFSH and LoFSH groups.

Variable	HiFSH	LoFSH
Age at castration	435 ± 2	438 ± 3
Body wt (kg)	147.2 ± 4.6	148.5 ± 5.8
Paired testes wt (g)[a]	314 ± 39	524.6 ± 48
Paired epididymal wt (g)[a]	96 ± 7.7	132 ± 9.5
Relative testes wt (g/kg body wt)[a]	2.15 ± 0.26	3.55 ± 0.33
DSP/g ($\times 10^{-6}$)[a]	22.0 ± 1.39	31.0 ± 1.72
TDSP/boar ($\times 10^{-9}$)[a]	6.55 ± 1.05	14.95 ± 1.30

[a] Groups differ; $p < 0.01$.

RESULTS

Concentrations of FSH and LH were lower ($p < 0.01$) in boars of the LoFSH group compared to the HiFSH group (Fig. 1). Boars in the LoFSH group had heavier ($p < 0.01$; Table 1) testes and epididymides and greater ($p < 0.01$) daily sperm production per gram of testicular tissue (DSP/g) and TDSP than boars from the HiFSH group. Surprisingly, plasma testosterone concentration was greater ($p < 0.01$) in boars of the LoFSH (4.7 ± 0.3 ng/ml) compared to those of the HiFSH (2.5 ± 0.2 ng/ml) group (Fig. 1). In contrast, incubation of testicular parenchyma indicated that there was no difference ($p > 0.10$) in total amount of secreted testosterone per paired testis or testosterone per 10^6 Leydig cells between boars of the HiFSH and LoFSH groups (Table 2).

Boars of the LoFSH groups had a greater ($p < 0.03$) volume percentage of tubules, a lesser ($p < 0.03$) percentage of interstitial cells, a lesser ($p < 0.03$) percentage of Leydig cells, and a tendency ($p = 0.06$) to have a reduced percentage of vasculature in the testis (Table 2) than boars of the HiFSH groups. The percentage of interstitial cells other than Leydig cells, the total number of Leydig cells, and the total volume of Leydig cells between the two groups were not different ($p > 0.78$).

The larger testes of boars of the LoFSH group contained a greater ($p < 0.03$) volume percentage of seminiferous tubules that were longer than ($p < 0.01$), but similar in diameter to, tubules in the smaller testes of boars from the HiFSH group (Tables 2 and 3). Volume of seminiferous tubules was greater ($p < 0.01$) in LoFSH than in HiFSH boars. The percentage of tubules that contained spermatids did not differ ($p > 0.80$) between boars of these two groups.

DISCUSSION

In the present study, boars with high systemic concentrations of FSH had smaller testes, a smaller proportion of their testes occupied by seminiferous tubules, and reduced DSP in comparison to boars with low systemic FSH. Meishan boars have much greater plasma FSH concentrations and much smaller testes than White Composite boars [4–6], but whether these were endocrine effects or inherent breed differences could not be determined. Thus, the use of crossbred boars in the current study provided animals of a similar genetic background, age, and body weight and established that high plasma FSH was associated with small testicular size.

Development of lumen in the seminiferous tubule is evidence of fluid secretion and establishment of the blood-

TABLE 2. Characteristics of testicular structures among boars in the HiFSH and LoFSH groups.

Variable	HiFSH	LoFSH
Volume % seminiferous tubules[a]	66 ± 2.6	77 ± 3.0
Volume % interstitial tissue[a]	34 ± 2.6	23 ± 3.2
Volume % Leydig cells[a]	19 ± 1.6	13 ± 2.0
Volume % vascular space[b]	8.6 ± 1	5.3 ± 1
Volume % other interstitial tissue	6.0 ± 1.2	5.4 ± 1.5
Calculated volume/Leydig cell (μm^3)	2029 ± 293	2142 ± 364
Calculated Leydig cell diameter (μm)	15.4 ± 0.8	15.9 ± 1.0
Total vol interstitium (cm^3/paired testes)	102.2 ± 16	123.5 ± 19
Total vol Leydig cells (cm^3/paired testes)	57.4 ± 8.4	67.4 ± 10.4
Total no. Leydig cells (10^{10}/paired testes)	3.08 ± 0.56	3.31 ± 0.69
Total testosterone (μg/paired testes/hr)	211.2 ± 83.5	271.9 ± 103.5
Testosterone (ng/10^6 Leydig cells/hr)	6.4 ± 2.6	8.4 ± 3.3

[a] Groups differ; $p < 0.03$.
[b] Difference between groups approached significance; $p = 0.06$.

testis barrier [21, 22]. Sertoli cell proliferation stops after the formation of the blood-testis barrier [23], and in European breeds of boars, this barrier is established around 90–120 days of age [24, 25]. In comparison, Meishan boars establish their blood-testis barrier at a much younger age, i.e., about 40 days of age [2]. Meishan boars have greater concentrations of FSH, LH, thyroid-stimulating hormone, and testosterone as compared to boars common to US producers [2–4, 26]; and concentration of FSH was negatively correlated with testicular weight within lines of boars of differing genetic backgrounds [10]. Thus, greater concentrations of these hormones in blood plasma at an earlier stage of prepubertal development may explain an earlier cessation of Sertoli cell proliferation and testicular growth in boars that have high systemic concentrations of FSH. However, HiFSH boars had higher plasma FSH concentrations at 2 and 8 wk of age [10]—a direct and unexplainable contradiction to FSH stimulation of Sertoli cell proliferation. Thus, some factor(s) other than the pituitary glycoproteins may limit testicular growth in HiFSH boars. Intratesticular hormones such as activin are likely candidates for consideration [27].

The current consensus is that testosterone is the primary hormone required to maintain spermatogenesis in adult rats [28]. The initial wave of spermatogenesis in pubertal rats is considered to require both LH and FSH support [29], but the role of FSH in spermatogenesis is not fully understood [30]. Boars in the HiFSH group had decreased TDSP compared to boars in the LoFSH group. This was predicted because TDSP is directly correlated with testicular size of boars [10, 19, 31]. Boars in the LoFSH group had a greater volume percentage of seminiferous tubules and greater length of tubules, which when combined, resulted in a greater total volume of tubules than for the boars of the HiFSH groups (Table 3). Boars of the two groups had similar seminiferous tubule diameter. Okwun et al. [6] reported a length of 1.78 km for seminiferous tubules in Meishan boars, which compares to a length of 1.74 km in boars of the HiFSH group. These values were much less than the 6.3 km in seminiferous tubule length reported by Bascom and Osterud [32] and 6.26 km reported by Okwun et al. [5, 6] for boars of White Composite breeding. Our observations support the suggestion of Bascom and Osterud [32] that length of the seminiferous tubules and not the diameter is important in determining total tubular volume and testicular size.

Additionally, boars in the HiFSH group had less DSP/g of testes compared to the boars in the LoFSH group; this observation contrasts with results obtained from comparisons of Meishan boars to those of White Composite breeding [10]. Although Meishan boars had less of their testes occupied by seminiferous tubules, they maintained similar DSP/g by having more germ cells per Sertoli cell and fewer germ cells lost during spermatogenesis [5].

Boars in the HiFSH group had a greater ($p < 0.03$) volume percentage of interstitial and volume percentage of Leydig cells, but total volume (cm^3/paired testes) of interstitium and Leydig cells was not different between boars of the HiFSH and LoFSH groups. The finding that boars in the LoFSH group had greater concentrations of testosterone in blood plasma than boars of the HiFSH group is intriguing because they had lower plasma LH concentrations than the HiFSH boars, and testosterone production in vitro was not different between boars of the two groups. Boars of the HiFSH group may have higher intratesticular concentrations of androgen-binding proteins or an increased rate of degradation of testosterone, which in turn would reduce concentrations of this hormone in blood plasma. Alternatively, there may be reduced efficiency of signal transduction via the LH receptors in boars of the HiFSH groups. Further studies should be conducted to address these questions.

Okwun et al. [6] reported in boars and VanDemark [33] in bulls that body weight and testis weight were highly ($p < 0.01$) correlated ($r = 0.94$, $r = 0.90$, respectively). These previous studies examined males across breeds that differed in mature body weight; in contrast, in the present study with genetically similar boars, there was no significant correla-

TABLE 3. Characteristics of seminiferous tubules among boars of HiFSH and LoFSH.

Items	HiFSH	LoFSH
Diameter of seminiferous tubules (μm)	393 ± 15	372 ± 19
Tubular length (km/paired testes)[a]	1.74 ± 0.28	3.71 ± 0.35
Tubular volume (cm^3/paired testes)[a]	211.4 ± 31	401.1 ± 38
Volume % of tubules occupied by lumen	23 ± 4	23 ± 5
% Seminiferous tubules with spermatids/total tubules	91 ± 3	89 ± 3

[a] Groups differ; $p < 0.01$.

tion between body weight and testis weight ($r = 0.06$). Thus, relative testes weight (testes weight/body weight) was less in boars of the HiFSH group (Table 1) and indicates that FSH-associated differences in testicular size occurred independent of body weight.

In conclusion, boars of similar genetic background that had greater concentrations of FSH had smaller seminiferous tubules (length and volume), which directly affected their spermatogenic capacity. This was substantiated by reduced daily sperm production per gram of testis and per boar in the HiFSH compared to the LoFSH group.

ACKNOWLEDGMENTS

We thank the Swine Operations Personnel for the care of the animals; S. Hassler, D. Griess, and A. Kruger for technical assistance; Dr. A.F. Parlow for LH antisera and pLH for iodination; and USDA/NIDDKD for FSH antisera, FSH and LH reference preparations, and oFSH for iodination.

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